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ANTIFUNGAL RESPONSE OF PHENOLS AND CRUDE EXTRACTS ISOLATED FROM SOME THELYPTEROID FERNS

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ABSTRACT

Crude extracts and extracted phenols from different plant parts (leaf, rhizome, petiole etc.) of some pteridophytes were tried experimentally to test their antifungal properties on a comparative basis. In this study, the antifungal properties of three members of the family Thelypteridaceae viz. *Ampelopteris prolifera*, *Christella dentata*, *Pronephrium nudatum* were tested against a pathogenic fungus *Aspergillus niger*. The amount of total free phenols from different parts of the above mentioned plants were measured. Presence of maximum amount of free phenols was noted in the sterile leaves and minimum in the rhizomes of all these thelypteroid members studied. *Pronephrium nudatum* showed accumulation of highest amount of free phenols in its sterile leaves and lowest accumulation was noted in the rhizomes of *Ampelopteris prolifera*. Ethanolic crude extracts and extracted phenols from all these plant parts showed antifungal effects.

Key words : *Antifungal response, Aspergillus niger, Crude extract, Extracted phenols, Thelypteridaceae*

Introduction

Non-flowering plants have been less exploited than flowering plants in the field of phytochemical analyses and medicinal uses. In recent days, ferns are gaining importance and they have been subjected to phytochemical and pharmacological analyses (cf. Irudayaraj and Patric Raja, 1998; Jesudass *et al*., 1993).

Phytochemical studies on ferns are extraordinarily important because of its immense economic values (Anderson *et al.*, 1979; Cui *et al.*, 1990), especially for their vast medicinal importance. Many ferns were used as medicines by early Greeks and Romans and also through the middle ages. Various workers have studied the pharmacology of several ferns like *Helminthostachys zeylanica* (Mitra *et al.*, 1973), *Marsilea diffusa* (Bharadwaja *et al*., 1977, 1983), *Polypodium vulgare* (Mannan *et al.*, 1989), *Dryopteris barbigera*, *D. cochleata*, *D. cryosocoma* and *D. splendens* (Mehra and Mittal, 1961a, 1961b, 1962; Yadav, 1997), *Lygodium flexuosum* (Khare and Tripathy, 1997), *Pronephrium nudatum* (Khare and Yadav, 1997) *Blechnum orientale* (Tripathy, 1997).

Ferns synthesize different kinds of phenolic compounds which include hydroxybenzoic acid, acyclophloroglucinols, many types of flavonoids etc. (Swain and Cooper-Driver, 1977)

From time to time many authors had worked on fern phenolics (Bohm and Tryon, 1967; White and Towers, 1967; Glass and Bohm, 1969; Bate and Smith, 1962, 1968; Wallace *et al*, 1984; Shankar and Khare, 1985, 1986; De-Britto *et al.*, 1992;

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Gopalkrishnan *et al.*, 1993; Sharma *et al.*, 1995; Kumar, 1995; Alonso- Amelot *et al.*, 2004; Guha *et al.*, 2004, 2005; and Ganguly *et al.*, 2011).

Phenolic compounds in plants act by selective blocking of infection (Barry *et al.*, 2002), reduced herbivore references (Bemays and Chamberlain, 1980; Boettcher and Targett, 1993; Coley and Barone, 1996; Lill and Merquis, 2001; Ossipov *et al.*, 2001; Ollerstam *et al.*, 2002; Remond *et al.*, 2002) and in allelopathy (Storey, 1991; Inderjit, 1996).

Earlier, accumulation of phenolic substances by different plant parts were noted by a number of workers (Rathore and Sharma, 1990, 1991; Guha *et al.*, 2005; Ganguly *et al.*, 2011).

Present study was undertaken to understand the comparative efficiency of antifungal activities of extracted phenols and crude extracts of different sporophytic plant parts of some members of Thelypteridaceae growing in West Bengal.

Material and Methods

Among the test plants, *Christella dentata* (Forssk.) Brownsey and Jermy and *Ampelopteris prolifera* (Retz.) Copel. were collected locally from Golapbag, Burdwan. *Pronephrium nudatum* (Roxb. ex Griff.) Holttum was taken from our departmental net house which is being maintained there from the original collection made from Sevak, Darjeeling, West Bengal. *Aspergillus niger* was taken as the test fungus to establish the antifungal activities of different sporophytic plant parts of the above mentioned plants.

To prepare both crude extracts and extracted phenols, 50 mg. of different sporophytic plant parts, viz. sterile leaf, petiole and rhizome were taken. In all cases, fresh plant materials were collected in the summer. Crude extracts were prepared by crushing each 50 mg. of the samples with 80% boiled ethanol in mortar and pastel. Each mixture was centrifuged at 4000 rpm for 10 minutes. The supernatant was taken and the volume was made up to 5 ml with 80% boiled ethanol. Now, 4 ml distilled water was added to that and the set was kept on a hot plate at 40°C for complete evaporation of ethanol.

In case of extracted phenols also, 50 mg. of different plant parts were taken and crushed with 80% boiled ethanol. The rest of the experiment to extract total free phenols was done by following the method of Bray and Thorpe (1954).

Preparation of potato dextrose agar medium (PDA):

Requirements:

Potato extract : 200-250 g
Dextrose : 20 g
Agar : 20 g
Distilled water : 1 ltr.

Preparation : Potatoes were cut into small pieces. 200-250 g of potato pieces were taken and boiled in distilled water for sometime until the potatoes become soft. Then the potato extract was filtered with the help of absorbent cotton and funnel. The diced potatoes were removed. After extraction the volume was made up to 1000 ml using distilled water. Again the extract was boiled and 20 g of dextrose was added into the extract. After sometime 20 g of the agar was mixed into the extract. It was then allowed to boil for sometime. Streptomycin strain was added into the extract to avoid bacterial contamination. pH of the extract was maintained between 6-6.5. The mixture was poured into the conical flask and the mouth of the conical flask was closed using cotton and brown paper.

The flask was then autoclaved for the purpose of sterilization.

Food poisoning technique : This was done following the methods mentioned by Mondal *et al.*, (1995). 20 ml of potato dextrose agar

(PDA) medium was poured in sterilized Petri dishes along with 1 ml of plant extract. The extract was mixed thoroughly with the PDA media by shaking in clockwise and anti-clockwise directions. The plates were allowed to be solidified. Then they were chilled for 1 hr. at 10°C. 5 mm diameter cups of PDA were removed from the centre of the Petri plates with the aid of a cork borer. The centers were then filled with the same diameter of mycelial dishes from young cultures (7 days from the day of inoculation). The Petri plates were wrapped with brown paper and incubated at 30°±1°C for 48-72 hrs. Colony growths were measured on the basis of circular dimensions.

Result and Discussion

Estimations of total free phenols of different parts of *Chistella dentata, Ampelopteris prolifera* and *Pronephrium nudatum* were done by measuring Optical Density (OD) in Systronics 117 UV-Vis Spectrophotometer at 650 nm and by putting the OD value in the following Arnon's (1949) principles. For each plant parts, three sets of data were taken along with a control set with distilled water and the average value was calculated.

The amount of total free phenols (mg/g fresh weight) in the three test plants are mentioned in Table 1.

TABLE 1— ESTIMATION OF FREE PHENOLS FROM DIFFERENT PLANT PARTS OF THE TEST PLANTS

Plants	Plant parts	Free Phenols (mg/g fresh wt.)
Christella dentata	Sterile leaf (Dil.-1:9)	3.22
	Petiole (Dil.-1:9)	2.62
	Rhizome (Without dil.).	1.30
Pronephrium nudatum	Sterile leaf (Dil.-1:9)	5.15
	Petiole (Dil.-1:9)	2.29
	Rhizome (Without dil)	1.42
Ampelopteris prolifera	Sterile leaf (Dil.-1:9)	2.20
	Petiole (Dil.-1:9)	1.99
	Rhizome (Without dil)	1.10

From Table 1, it is clearly observed that in *Pronephrium nudatum*, sterile leaf accumulates the maximum amount (5.15 mg/g fresh wt.) of total free phenols while the rhizome accumulates the lowest amount (1.30 mg/g fresh wt.). Similar observations have been found in case of *Christella dentata and Ampelopteris prolifera.*

Measurements of fungal growth area in response to crude extract and extracted phenols of different plant parts of previously mentioned three pteridophytic species against *Aspergillus niger* are given in Table 2 and Table 3.

TABLE 2 — MEASUREMENTS OF FUNGAL GROWTH AREA BY CRUDE EXTRACTS OF DIFFERENT PLANT PARTS IN RESPONSE TO *ASPERGILLUS NIGER*: GROWTH OF THE PATHOGEN IS EXPRESSED IN TERMS OF SQ. CM.

Plants	Plant parts	Volume of extarcts (1ml) (Fungal growth in sq. cm)	Volume of extarcts (1.5ml) (Fungal growth in sq. cm)	Volume of extarcts (2 ml) (Fungal growth in sq. cm)
Pronephrium nudatum	Sterile leaf	15.20	10.50	9.90
	Petiole	16.00	15,05	12.95
	Rhizome	24.80	18.00	16.00
Christella dentata	Sterile leaf	5.40	-	4.20
	Petiole	5.1	-	3.90
	Rhizome	7.4	-	4.00
Ampelopteris prolifera	Sterile leaf	76.40	68.20	52.88
	Petiole	78.98	72.00	59.94
	Rhizome	79.68	75.33	62.32

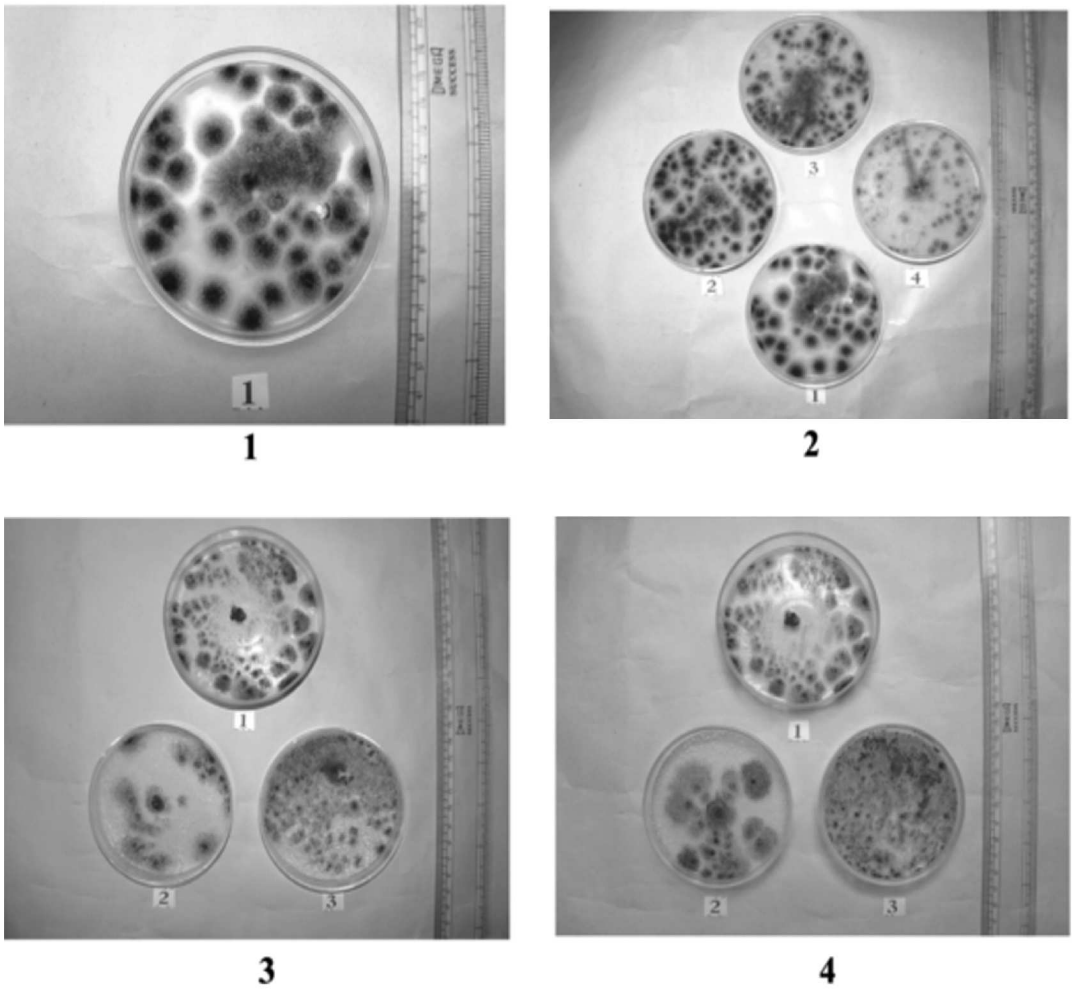
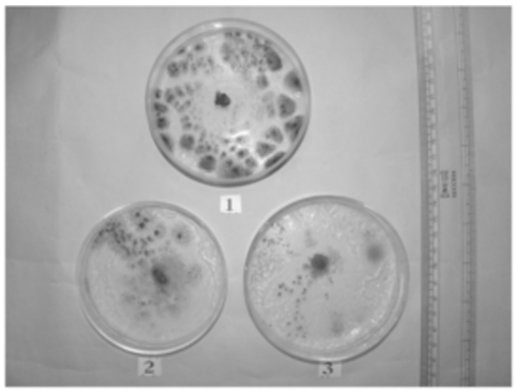


Fig. 1. Growth of *Aspergillus niger* under controlled condition.

Fig. 2. Zone of inhibition of *Aspergillus niger* by crude ethanolic extracts of sterile leaf of *Ampelopteris prolifera*. 1=control, 2=1 ml, 3=1.5 ml, 4=2 ml.

Fig. 3. Zone of inhibition of *Aspergillus niger* by extraced phenol from petiole of *Amelopteris prolifera*. 1=control, 2=2 ml, 3=1 ml.

Fig. 4. Zone of inhibition of *Aspergillus niger* by extraced phenol from sterile leaf of *Amelopteris* prolifera. 1=control, 2=2 ml, 3=1 ml.



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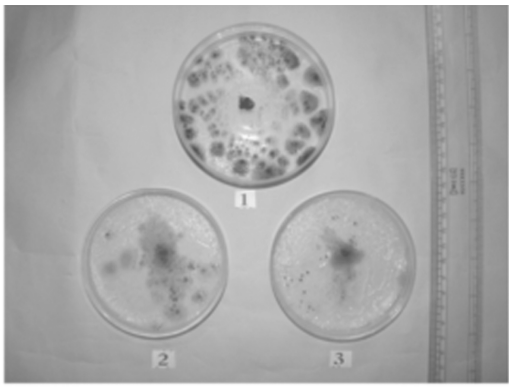

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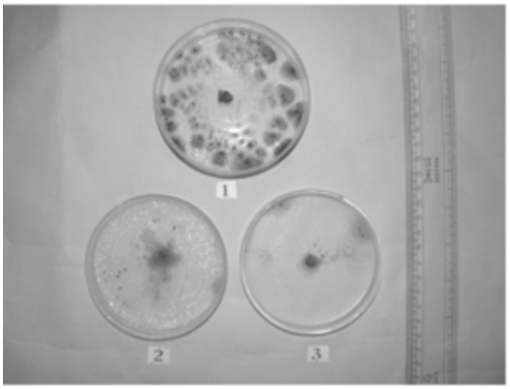

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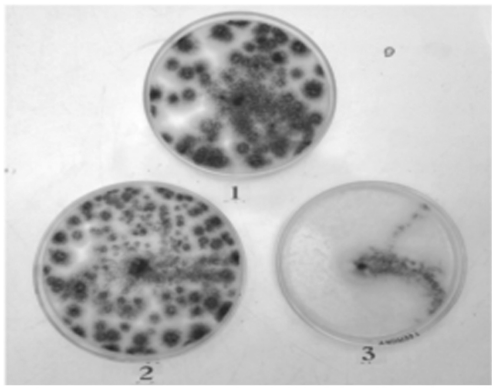

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Fig. 5. Zone of inhibition of *Aspergillus niger* by extracted phenol from rhizome of *Ampelopteria Prolifera.* 1=control, 2=1 ml, 3=2 ml.

Fig. 6. Zone of inhibition of *Aspergillus* niger by crude ethanolic extracts of rhizome of *Christella dentata*. 1=control, 2=1 ml, 3=2 ml.

Fig. 7. Zone of inhibition of *Aspergillus* niger by crude ethanolic extraced of petiole of Christella dentata. 1=control, 2=1 ml, 3=2 ml.

Fig. 8. Zone of inhibition of *Aspergillus* niger by extraced from petiole of *Pronephrium nudatum*. 1=control, 2=1 ml, 3=2 ml.

TABLE 3. MEASUREMENTS OF FUNGAL GROWTH AREA BY EXTRACTED PHENOLS OF DIFFERENT PARTS IN RESPONSE TO *ASPERGILLUS NIGER*. GROWTH OF THE PATHOGEN IS EXPRESSED IN TERMS OF SQ. CM.

Plants	Plant parts	Volume of extract (1 ml) (Fungal growth in sq. cm)	Volume of extract (2 ml) (Fungal growth in sq. cm)
Pronephrium nudatum	Sterile leaf	64.00	18.04
	Petiole	72.00	22.00
	Rhizome	74.80	20.25
Christella dentata	Sterile leaf	31.05	22.62
	Petiole	35.70	27.60
	Rhizome	17.20	11.20
Ampelopteris prolifera	Sterile leaf	76.26	18.75
	Petiole	59.20	21.40
	Rhizome	29.00	26.40

After detailed study of antifungal effect (using Food Poisoning Technique), it was revealed that all these three test plants show inhibitory effects against the growth of the pathogenic fungus *Aspergillus niger*. In case of crude extracts, 2 ml water extract of sterile leaf of *Christella dentata* was proved as the most effective. Regarding extracted phenol, *Ampelopteris prolifera* showed significant results. In all the cases, 2 ml of the volume of both crude extracts and extracted phenols have been proved as much more effective than 1 ml. *Pronephrium nudatum*, in both cases (Crude extract and extracted phenol), showed significant result in respect to it's leaf. In case of *Christella dentata,* petiolar crude extract showed maximum inhibition, regarding extracted phenol, rhizome showed the maximum inhibition. In case of *Ampelopteris prolifera*, crude extract of sterile leaf showed maximum inhibitory result, regarding antifungal effects by extracted phenols, rhizome gave the maximum inhibition.

From the above results it is revealed that, the plant extracts of the four test plants have significant inhibitory activity against *Aspergillus niger*. Crude extracts of the plant parts are more potential than their extracted phenols regarding antifungal property. It is probably due to the cumulative effect of some other unknown compounds along with phenolic compounds. Antimicrobial property of this plant might be attributed to the presence of high levels of phenolic compounds (Guha *et al*., 2005).

Current microbial resistance to antibiotics has been creating a global problem to the scientists. So, there is an urgent need of new compounds for antimicrobial therapy. It has been found that phenolic compounds in plants have strong antimicrobial properties against fungal infections. Pharmacological, pharmaceutical, phytopathological and food processing industries are some of the fields where phenolic compounds can be applied as bio- preservatives.

Conclusion:

From the above results we can conclude that these three thelypteroid pteridophytes have the potentiality to establish themselves as ethno-pharmacological antifungal agents. However, antifungal effects with some other human pathogenic fungi are in process to confirm the spectrum of efficacy of these plants.

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